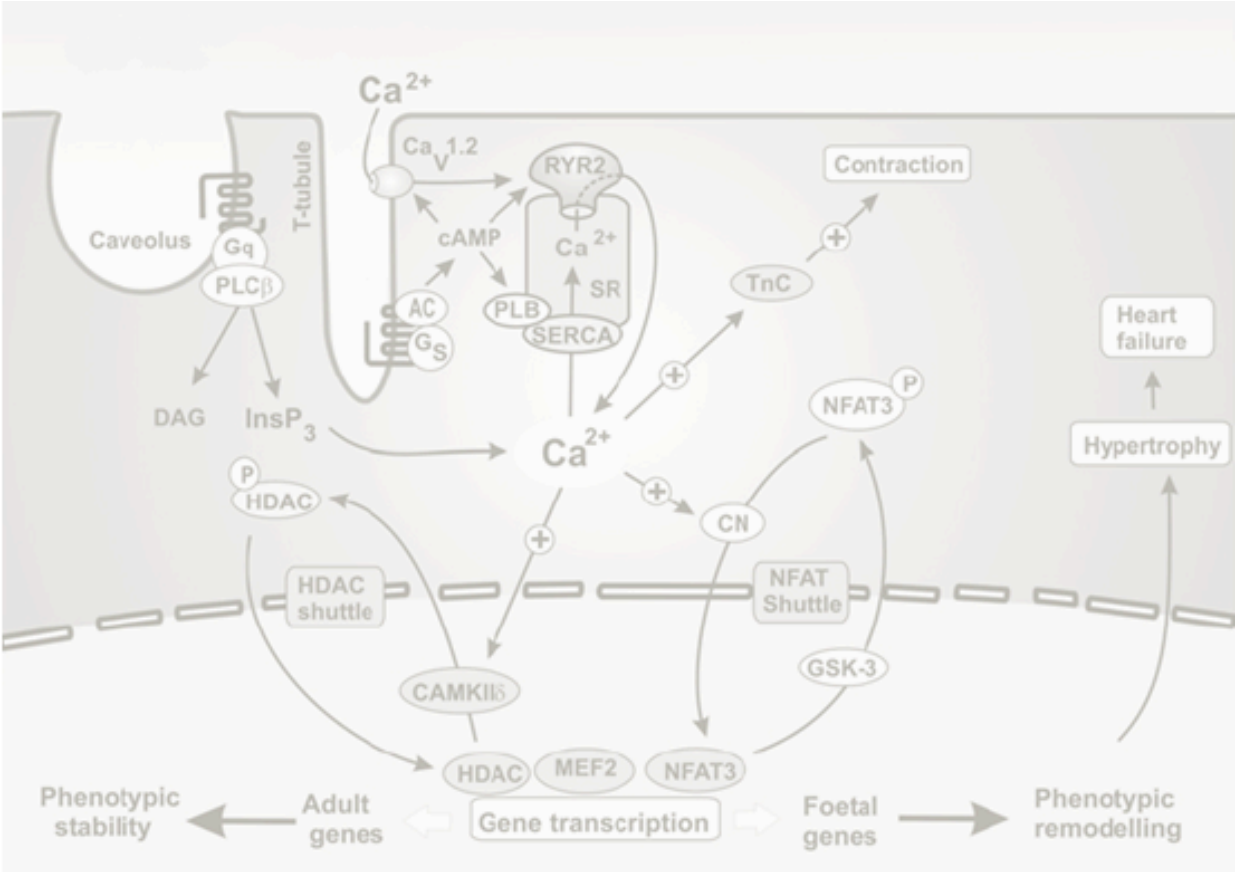
Calcium signalling in the heart

From contraction to gene transcription



Thesis Master Biology of Disease

University of Utrecht

Rianne A.M. Bouwmeester

3330303

Supervisor: T.P. de Boer PhD

University Medical Center Utrecht

Department of Medical Physiology

Content

Cover: Adapted from Berridge (2006).

Abbreviation list:

Ca^{2+}	Calcium
$[Ca^{2+}]_x$	Calcium concentration in a certain region
CaM	Calmodulin
CaMK	CaM kinase
CICR	Calcium induced calcium release
Cn	Calcineurin
Cq	Calsequestrin
CRAC	Calcium release-activated channel
CREB	cAMP response element-binding, is a transcription factor.
CPB	CREB binding protein
DAG	Diacylglycerol
EC-coupling	Excitation-contraction coupling
ER	Endoplasmic reticulum; in non-contractile cells
ET-coupling	Excitation-transcription coupling
HF	Heart failure
IP_3	Inositol 1,4,5-Triphosphate
IP_3R	IP_3 receptor
JCN	Junctin
MCU	Mitochondrial Ca^{2+} Uniporter
NAADP	Nicotinic acid adenine dinucleotide phosphate
NCX	Na^+/Ca^{2+}-exchanger
NE	Nuclear envelope
NFAT	Nuclear factor activated T-cells
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B-cells; TF
PKA	Protein kinase A; cAMP dependent enzyme
PKC	Protein kinase C; activated by increase [DAG] or $[Ca^{2+}]$
PLB	Phospholamban, inhibitor of SERCA activity
PLC	Phospholipase C; activated by G-protein coupled receptors to adenylyl cyclase to generate cAMP.
PMCA	Plasma membrane Ca^{2+} ATPase
PTM	Post translational modifications
RyR	Ryanodine receptor
RTK	Receptor tyrosine kinase
SERCA	SR/ER Ca^{2+} ATPase
SR	Sarcoplasmic reticulum; in muscle cells
TF	Transcription factor
TRD	Triadin

Abstract:

The human heart contracts approximately 75 times per minute under resting conditions. These contractions are mediated by fluctuations in cytosolic Ca^{2+} concentration. In heart failure the cardiomyocytes are not able to handle these Ca^{2+} signals properly. As a consequence the heart starts to remodel, a process initiated by altered gene transcription. The regulation of these processes is mediated by one and the same signalling ion, i.e. Ca^{2+}. This review will discuss Ca^{2+} handling mechanisms ranging from cytosolic Ca^{2+} homeostasis and the regulation of gene expression to their role in heart failure.

Introduction

To be able to deliver oxygen to all organs, blood needs to be transported throughout the body. The circulation of blood is mediated by the rhythmic contractions of the heart. Under resting conditions the human heart beads approximately 75 times per minute.

The cardiac rhythm is initiated by action potentials generated in the SA node that trigger voltage dependent, L-type Ca^{2+} channels (LTCC). Opening of these channels causes Ca^{2+} to enter the cytosol. Subsequently, the increased intracellular Ca^{2+} concentration is sensed by Ryanodine receptors (RyR), which open their Ca^{2+} release channels in the sarcoplasmic reticulum (SR) allowing significantly more Ca^{2+} to enter the cytoplasm. This mechanism is called Ca^{2+} induced Ca^{2+} release (CICR). The released Ca^{2+} ions directly activate myofibrils as they bind to myofilament protein Troponin C. Troponin C functions as a Ca^{2+} binding protein that controls other proteins under which Tropomyosin, the blocker of myosin binding sites on actin filaments. Upon Ca^{2+} stimulation Troponin C induces a conformational change in Tropomyosin, thereby unmasking these myosin binding sites. Due to cross-bridging of actin and myosin, a process energized by the hydrolysis of ATP, physical contraction is induced. This whole process is termed excitation-contraction (EC) coupling. [1]. Key players in EC-coupling are illustrated in figure 1.

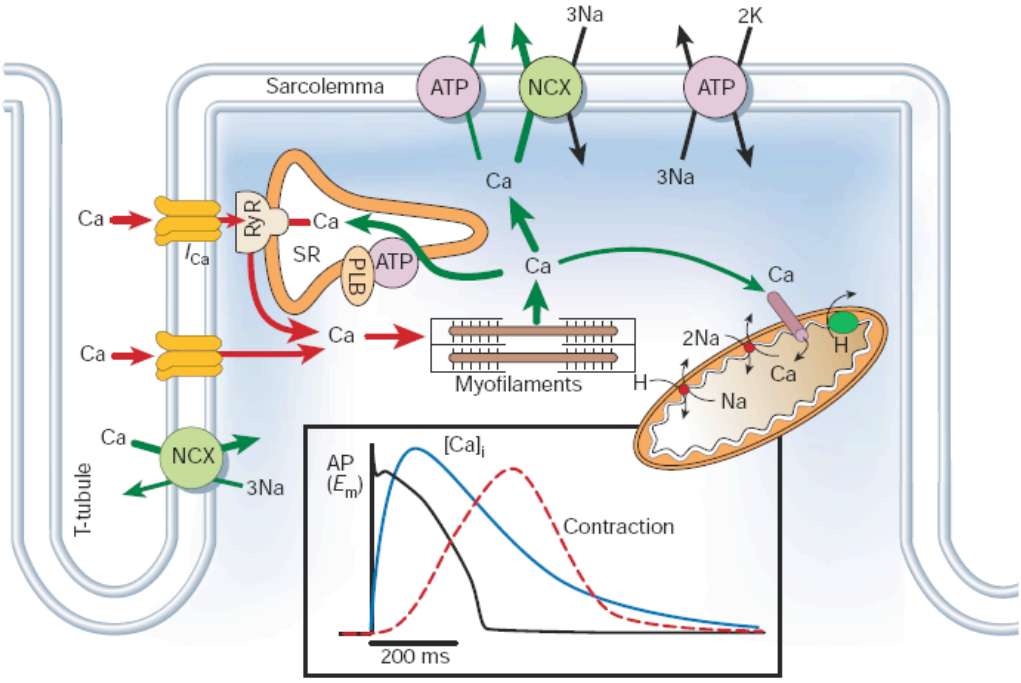


Figure 1: Overview of Ca^{2+} pathways and transporters in EC-coupling. The inserted graph illustrates the timeline and slope of an action potential (AP), intracellular Ca^{2+} concentration ($[Ca]_i$) and contraction of the heart. (Picture adapted from Bers et al[2]).

To end contraction (and allow relaxation of cardiomyocytes) Ca^{2+} ions must dissociate from Troponin C and be removed from the cytosol. This cytosolic decrease is mediated by five transporters knowing Sarco (Endo)plasmic Ca^{2+}ATPase (SERCA), Na^{+}/Ca^{2+} Exchanger (NCX), Sarcolemma Ca^{2+} ATPase (SCA), the secretory pathway Ca^{2+} ATPase (SPCA) and the mitochondrial uniporter (MCU)[2]. (CICR and the main Ca^{2+} transporters will be further discussed below).

Besides removal of cytosolic Ca^{2+}, must RyR channels be closed. To this end Zima et al. demonstrated that Ca^{2+} release is robustly stopped when the SR Ca^{2+} content reaches an apparent threshold of 60% of diastolic $[Ca^{2+}]_{SR}$ under resting conditions [3].

Heart failure

Heart failure (HF) is one of the major causes of human morbidity and mortality. During HF cardiomyocytes have a reduced ability to perform their function [4]. One of the central causes of HF is the incorrect handling of Ca^{2+} by cardiomyocytes resulting in contractile dysfunction and arrhythmias [2]. Incorrect Ca^{2+} handling can be caused by alterations in the Ca^{2+} amplitude, the duration of the Ca^{2+} transient or decreased myofilament sensitivity due to acidosis, elevated phosphate or Mg^{2+} concentrations (*i.e.* measurable parameters in ischemia).

The duration of Ca^{2+} transients can be affected by changes in the open probability of RyRs. This open probability of RyR is known to be increased when the Ca^{2+} load in the SR ($[Ca^{2+}]_{SR}$) is high and could therefore induce spontaneous SR Ca^{2+} release. A phenomenon known to trigger arrhythmias because cytoplasmic release of Ca^{2+} can cause activation of the electrogenic NCX in the plasma membrane. Moreover, caffeine and certain inotropic drugs are known to increase myofilament sensitivity [2], due to their stimulating effect on these RyRs [5,6].

The amplitude of Ca^{2+} transients is known to be indirectly altered due to changes in the expression of Ca^{2+} handling genes. Normally approximately 70% of the $[Ca^{2+}]_i$ is removed by SERCA, ~28% via NCX and the remainder via the slow systems (i.e. sarcolemma Ca^{2+} ATPase and MCU (numbers correspond to human and rat)) [7,8]. During HF, the functional expression of NCX is increased whereas SERCA is dramatically reduced [9]. As a consequence the Ca^{2+} load in the SR is reduced [10-12], thereby limiting Ca^{2+} release in processes such as CICR. Moreover the reduced expression of SERCA is known to be accompanied by a downregulation of RyRs [13]. The reduced $[Ca^{2+}]_{SR}$ is considered to be the central cause of contractile dysfunction during HF [14].

One ion: many functions.

As described above, Ca^{2+} ions are involved in many cellular processes. How is it possible that one and the same ion is able to fulfill multiple roles within the same cell? How are Ca^{2+} ions used to induce both rhythmic contraction of cardiomyocytes and regulate transcription of specific genes at the same time?

What are the key players in Ca^{2+} homeostasis and which organelles are involved? Is the intracellular localization of these ions of special importance? Are the induced responses a direct effect of this ion, or are there intermediates that do the job? Are there stimulatory or co-activating proteins involved in these mechanisms? What is the main purpose of Ca^{2+} in the cytosol and what is it in the nucleus? Are these mechanisms involved in heart failure?

The current review aims to clarify the regulation of Ca^{2+} concentrations and localization within the cell. The main transport proteins, Ca^{2+} release mechanisms, their inhibitors and stimulators will be discussed briefly, as well as the role of Ca^{2+} in gene expression.

As an example of Ca^{2+} induced gene transcription, the NFAT-pathway from Ca^{2+} signal towards gene transcription will be explained. The most common consequences of improper handling of Ca^{2+} in heart failure are described.

Calcium homeostasis in the cytosol.

Ca^{2+} release mechanism: CICR

Once the action potential induces a depolarization of the plasma membrane LTCCs open. The entry of Ca^{2+} is sensed by binding of 4 Ca^{2+} ions to the RyR that extends through the SR membrane. Neighboring RyRs subsequently open their channels due to the local, high [Ca^{2+}] or due to coupled gating [2]. During this process of Ca^{2+} induced Ca^{2+} release (CICR) the Ca^{2+} concentrations of approximately 100 nM in resting cardiomyocytes, raises to 1 or 2 µM during the action potential [15].

In the heart RyRs form tetramers, in which each RyR is bound to a FK506-binding protein (FKBP12). FKBPs are known to diminish RyR channel activity [16]. Therefore blockage of the FKBP binding site results in increased Ca^{2+} release from the SR [15].

Furthermore RyRs form complexes with Triadin, calsequestrin and Junctin on the luminal side of the SR membrane. Together they sense and translate changes in luminal $[Ca^{2+}]_{SR}$ to RyR_2 [17]. TRD acts as a scaffolding protein that concentrates calsequestrin (Cq) near the junctional phase in the SR, thereby enabling the RyR to sense and response to changes in $[Ca^{2+}]_{i.}$ However, others state that TRD directly regulates RyR channel activity [18].

Three isoforms of TRD have been found in human, from which TRD-1 (40 kDa) is most abundant in heart [19]. Interaction with RyR-complexes occurs via the C-tail of TRD [17,19], which is characterized by a KEKE motif (long stretches of alternatively positive or negatively charged residues) that is considered to be the protein-protein binding domain. This interaction does not depend on charge or Ca^{2+}, in contrast to interaction between TRD and Cq [20]. By concentrating Cq near the RyRs or due to a direct regulation of the channel activity, Ca^{2+} release is facilitated [17,20]. More details concerning the regulatory effects of the Ca^{2+} buffering protein Cq will be given below when the major uptake system SERCA is discussed.

Junctin (JNC) is a 26 kDa Cq binding protein localized in the SR membrane [21,22]. JCN and TRD have a similar topology, i.e. a cytosolic N-terminus, a single membrane spanning domain and a highly charged C-terminus at the luminal side of the SR. This luminal domain comprises a KEKE motif and is therefore a potential site for protein interaction. JCN, like TRD, is suggested to modulate RyR activity [18] and may affect the SR Ca^{2+} load. Only the major difference between these two regulatory effectors is that JCN contains multiple Cq binding domains in contrast to a single binding site in TRD [20].

Besides activation by Ca^{2+} entering the cell through the LTCC in CICR, RyR activation is induced by stimulation with cyclic ADP-ribose (cADPR) or nicotinic acid adenine dinucleotide phosphate (NAADP) [23]. cADPR and NAADP are products from the same ancestor i.e. beta-NAD^+, which itself is not able to activate RyRs [24].

Sufficient levels of cADPR have mainly been found in non-RyR expressing cells, indicating that cADPR might as well be regarded as a relatively constant regulator of Ca^{2+} signaling in different cell types [24]. Whereas NAADP is thought to have either a direct effect on type 1 RyRs (at least in muscle cells) or via a tightly bound protein [25]. NAADP was first discovered in sea urchin eggs

[26,27] but the exact mechanism of action and receptor for this signaling molecule are still under investigation [24].

Besides NAADP and cADPR, also inositol-1,4,5-triphosphate (IP_3) is able to trigger release of Ca^{2+} from the SR [23]. IP_3 (and diacylglycerol (DAG)) are generated by PLC through the hydrolysis of PIP_2. Both metabolites (IP_3 and DAG) are able to activate PKC. As a consequence, PKC is transferred towards the plasma membrane where it is (indirectly) involved in receptor desensitization, in modulating membrane structure events and regulating cell growth and transcription. Because PKC effects are indirect, the consequences of activation might differ between cell types.

The identification of IP_3 induced Ca^{2+} release was done by Berridge and coworkers [28]. Later it was shown that IP_3 is able to trigger Ca^{2+} release by interacting with the IP_3 receptor (IP_3R), which is structurally and functionally analogous to the RyR [29]. Both receptors are Ca^{2+} channels that facilitate transduction of extracellular stimuli into cytosolic Ca^{2+} signals [30], although action potentials are not known to stimulate IP_3 production [2].
Binding of IP_3 to the IP_3R initiates a conformational change, due to which the integral channel opens and Ca^{2+} ions enter the cytosol [24]. Although opening of IP_3Rs requires IP_3 binding, channel activation is fine-tuned by the cytosolic [Ca^{2+}] [31]. As a consequence Ca^{2+} release via IP_3Rs (and RyRs) has a positive feedback on its own [32]. Inhibition of IP_3 induced Ca^{2+} release is subsequently caused by the reduced $[Ca^{2+}]_{SR}$ [33] as well as the increasing concentration of Ca^{2+}/CaM complexes in the cytosol [34, 32].

Of note, the action of IP_3 is most likely restricted to a small area, as its lifetime is shortened by fast metabolization by enzymes which either add or remove phospho-groups [30]. An example is the addition of a phospho-group by Ca^{2+}-dependent kinase, thereby generating IP_4. This metabolite is able to bind to GTPase-activating proteins of the Ras family that are known to modulate Ca^{2+} release [35].

Ca^{2+} uptake mechanisms

In order to maintain a relatively constant [Ca^{2+}] in the cytosol, Ca^{2+} homeostasis requires both Ca^{2+} release (via CICR) and Ca^{2+} uptake. The decrease of cytosolic Ca^{2+} levels is mediated by five major pathways involving Sarco (Endo)plasmic Ca^{2+}ATPase (SERCA), Na^{+}/Ca^{2+} Exchanger (NCX), mitochondrial uniporter (MCU), the Sarcolemma Ca^{2+} ATPase (SCA) and a golgi specific ATPase (SPCA) [2]. The main properties of these transporters will be discussed briefly.

SERCA

SERCA is a P-type ATPase, that fulfills the adverse mechanism of CICR as it is responsible for the active uptake of Ca^{2+} in the SR, the golgi complex as well as in the nuclear envelope. A SERCA monomer consists of one peptide chain folded into four domains; a transmembrane domain containing 10 transmembrane helices and both Ca^{2+} binding sites, and three cytosolic domains composed of the nucleotide binding domain and 2 actuator domains. These actuator domains contain SERCA's phosphorylation sites [36]. The Ca^{2+} binding sites in the transmembrane domain have a high affinity for Ca^{2+} when exposed to the cytosol (E1 state), whereas the affinity is low when facing the SR lumen (E2 state). Ca^{2+} uptake by SERCA is energized by the hydrolysis of ATP [37,38].

The activity of SERCA is regulated by two types of proteins, knowing Calsequestrin and phospholamban. Calsequestrin (Cq) stimulates SERCA activity, since it functions as a Ca^{2+} buffering protein that is able to bind up to 20 Ca^{2+} ions per molecule. The binding of Ca^{2+} to Cq is mediated by the electrostatic attraction to negatively charged pockets that are formed when Cq proteins oligomerize. Due to the high binding capacity of Cq, the free luminal [Ca^{2+}] is strongly reduced and consequently the Ca^{2+} gradients between the cytosol and storage organelles such as the SR are maintained [2].

Cq expression is regulated at transcriptional level by Early growth response-1 (Egr-1) [39] in a cell type and tissue specific manner. Egr-1 therefore binds to the promoter site of Cq, a site overlapping the putative NFAT binding site. Overexpression of Cq results in downregulation of several proteins involved in CICR such as RyR, TRD and JCN [2]. Cq is of special importance in cardiac cells, since mutations in Cq are associated with tachycardia, cardiomyopathies and sudden cardiac death [40] (involvement of Cq in heart failure is further discussed below).

Phospholamban (PLB) is the endogenous inhibitor of Ca^{2+} uptake as it reduces SERCA activity. PLB is a small (52 AA) type II membrane protein and is highly expressed in the SR [36]. A PLB monomer will preferentially bind to SERCA in the E2 state, thereby reducing the Ca^{2+} affinity of the pump. The inhibition of SERCA results in a decreased muscle relaxation and contractility rate, causing a decreased heart rate and stroke volume [2].

However this inhibitory effect on SERCA is reduced by phosphorylation of Ser16 by PKA, or Thr17 by CaMK type II [41]. Phosphorylation inactivates PLB and allows faster relaxation and declines the cytosolic [Ca^{2+}] as it increases the Ca^{2+} content in the SR [4]. Phosphorylation of PLB is by far the most effective way to accelerate cytosolic Ca^{2+} decrease [42] and results in a better competition between SERCA activity and transport via NCX [2].

NCX

The NCX (Na^+/Ca^{2+}-exchanger) is a secondary active transporter that exchanges three Na^+ ions for one Ca^{2+} ion. The exchanger is expressed at the plasma membrane, in the SR and in mitochondria [2]. Its activity is facilitated by the primary active transport of the Na^+/K^+-ATPase that normalizes the generated Na^+ gradient. It has been demonstrated by several groups that the activity of NCX is used to rapidly remove Ca^{2+} from cardiomyocytes [43-45].

The net direction of transport by NCX is mainly influenced by the Na^+ and Ca^{2+} gradient and the membrane potential. Because this transport is electrogenic, depolarization of the membrane might reverse the direction, although the physiological role of reversed transport is still unclear. It is known that NCX activity is stimulated upon phosphorylation, since DiPolo and Beauge demonstrated that Ca^{2+} dependent kinases are able to induce a reaction that results in the activation and increased affinity for cytoplasmic Ca^{2+} and extracellular Na^+ [46]. This experiment was preformed with squid giant axon, and has been difficult to reproduce in other tissues. However, similar indications have been obtained in cardiac and aortic smooth muscle [47,48].

NCX activity can be reduced via ATP-induced production of PIP_2 [49] since activation of PLC will decrease the membrane PIP_2 level and thereby inhibit the exchanger by increasing inactivation. This may subsequently lead to decreased Ca^{2+} extrusion, although this hypothesis requires testing [50].

Mitochondria (MCU)

Mitochondria are involved in Ca^{2+} homeostasis, not only due to the expression of NCX, but also by the expression of the Mitochondrial Ca^{2+} Uniporter (MCU). Ca^{2+} uptake by the MCU is driven by the negative membrane potential of mitochondria, which is normally between -150 and -180 mV [51]. The inner mitochondrial membrane is impermeable to Ca^{2+}, therefore uptake requires the activity of the MCU [52]. The MCU is a highly selective channel [53] with a low affinity but high Ca^{2+} capacity. Since there is no counter-ion in this type of Ca^{2+} transport, the mitochondrial membrane must depolarize during uptake [54]. The membrane potential is suggested to be restored due to an increased rate of ATP production by increased free mitochondrial Ca^{2+} [55].

Nevertheless (prolonged) Ca^{2+} overload in mitochondria must be avoided, because free luminal Ca^{2+} above a certain threshold will activate the permeability transition pores (PTPs) that are able to trigger cascades of cellular processes leading to apoptotic cell death [56]. PTPs are composed of multiple subunits forming a voltage dependent, non-selective channel spanning the inner and outer mitochondrial membrane [52]. Once the PTPs are activated, the membrane potential will collapse. As a consequence either PTPs open or the mitochondrial membrane locally disrupts, thereby releasing nucleotides, Ca^{2+} ions and several pro-apoptotic proteins including cytochrome C into the cytosol. However, PTPs also appear to have a small-conductance state and it is therefore believed that PTPs somehow participate in physiological Ca^{2+} handing in the form of CICR [57].

Golgi apparatus (SPCA)

Another important intracellular storage compartment is the golgi system. This system is responsible for the storage of approximately 5% of total cellular Ca^{2+} but in significantly higher concentrations (1-2 mM) [58]. The uptake of Ca^{2+} from the cytosol is mediated by two types of Ca^{2+} pumps: the classical SERCA pump (discussed above) and the secretory pathway Ca^{2+} ATPase (SPCA) [59,60].

The SPCA is very similar to SERCA but smaller and exclusively expressed in the golgi [61]. SPCA contains 10 TM domains, ATP and FITC binding sites and a conserved phosphorylation site in its actuator domain.

In human SPCA is encoded in two genes (ATP2C1&2) encoding SPCA 1 and 2 respectively [62]. Although all essential residues involved in Ca^{2+} pumping are conserved in these isoforms, it is currently not clear whether SPCA2 is able to transport Ca^{2+} and what its biological function might be. SPCA1 on the other hand is suggested to transport one cation at the cost of one ATP, since PMR1, the yeast homologue of SPCA1, contains one single Ca^{2+} binding site [63].

Besides Ca^{2+} is SPCA able to transport Mn^{2+} [59]. Sufficient levels of Mn^{2+} and Ca^{2+} are required for secretory pathways and correct glycosylation of secretory proteins [64] for which the *cis*-golgi is mainly responsible [59, 65]. Furthermore Mn^{2+} is an essential cofactor for a number of enzymes in the cytoplasm such as amino-peptidase P [66].

Decreasing $[Ca^{2+}]$ in the golgi, or actually increasing the cytosolic $[Ca^{2+}]$, is realized by rapid release via IP_3R localized in the *cis*-golgi [67]. No evidence for other release mechanism such as cADPR or NAADP, in this compartment have been found yet.

Extracellular storage (SCA)

In order to maintain homeostasis, Ca^{2+} ions originating from outside the cell, must be transported back into the extracellular fluid. Transport across the plasma membrane (sarcolemma) is mediated by a high affinity Ca^{2+} handling system, the plasma membrane Ca^{2+} ATPase ((PMCA) also named sarcolemma Ca^{2+} ATPase (SCA)). PMCA is besides SERCA and SPCA the third of three distinct classes of type II P-type Ca^{2+} ATPases identified in mammalian cells [68].

Due to its ubiquitous expression and low transport capacity, the PMCA is considered a housekeeping system responsible for setting and maintaining the normally low $[Ca^{2+}]_i$ [69]. PMCA is a P-type ATPase [70], with intracellular N and C termini, 10 transmembrane domains and two major cytosolic loops [71]. In human 4 major isoforms, encoded by separate genes, have been identified. However due to alternative splicing over 20 variants are known. These variants show their own developmental, tissue and cell-specific expression patterns [72]. Despite the high similarity among these variants, tail sequences are the least conserved domains [70]. Surprisingly because the C-tails are involved numerous protein-protein interactions, including binding of the major regulator CaM. PMCA activation requires Ca^{2+} /CaM binding to its C-terminus, inducing a conformational change, thereby displacing the auto-inhibitory tail from the cytosolic loops [73]. PMCA activity is furthermore affected by acidic phospholipid, partial proteolysis, phosphorylation and dimerization of the C-termini [74].

In summary; basically every intracellular organelle is in some way involved in cytosolic Ca^{2+} homeostasis. The sarcoplasmic reticulum is most likely the biggest player, since SERCA pumps are the major uptake mechanism, and CICR (mediated by IP_3R and RyRs) is the main release mechanism. SERCA or SERCA-like pumps are also found at the plasma membrane (PMCA) and in the golgi apparatus (SPCA). Again stimulation of the IP_3R is the main release mechanism in the latter compartment. The mitochondrial MCU as well as the NCX are of big importance in Ca^{2+} homeostasis (Ca^{2+} lowering mechanisms are depicted in figure 3). Together these Ca^{2+} transporters realize an almost stable long-term Ca^{2+} concentration within the cytosol and at the same time allow physiological processes such as EC-coupling in cardiomyocytes.

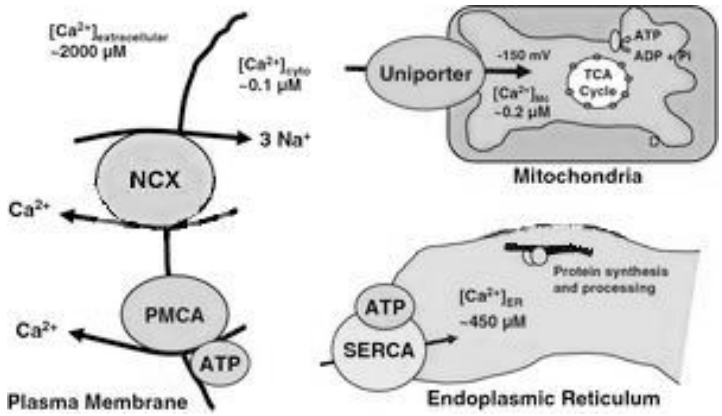


Figure 3: An overview of the 4 major Ca^{2+} lowering mechanisms.
SERCA transports Ca^{2+} by hydrolysis of ATP into the SR, mitochondria express MCU, the plasma membrane contains Na^{+}/Ca^{2+}-exchangers and the PMCA. Ca^{2+} uptake into the golgi apparatus is not indicated.

Calcium across the nuclear envelope.

The Ca^{2+} concentration in the nucleus ($[Ca^{2+}]_n$) is different compared to the cytosol. This difference is mediated by a barrier between these two compartments, called the nuclear envelope (NE) which is composed of a double membrane. Still, extracellular signals are able to eventually induce altered transcription of specific genes by changing the nuclear Ca^{2+} concentration. In cardiomyocytes the outer membrane is continuous with the SR [75], therefore the NE is suggested to function as a Ca^{2+} store as well [76-78].

There are two main pathways for Ca^{2+} to cross the NE, which are by active transport or via passive diffusion. Active transport across the NE requires uptake and release mechanisms that are localized in respectively the outer and inner membrane. Uptake of cytosolic Ca^{2+} is thereby mediated by the earlier discussed SERCA pump, which is activated by PKA phosphorylation [79]. According to Gensburger et al., SERCA pumps are accompanied by IP_4Rs [38], which may control an ATP-independent uptake mechanism [75,80].

Release of Ca^{2+} into the nucleoplasm is mediated by IP_3Rs and RyRs [81-83]. These receptors can be activated by their agonists IP_3 and cADPR [23,79], since the complete toolkit for the production of IP_3 [84] as well as enzyme required for NAD^+ cleavage, ADPribosyl cyclase, [85], exist in the nucleus [86].

Passive Ca^{2+} transport, i.e. diffusion, is mediated by nuclear pore complexes (NPCs) that span the inner and outer membrane of the NE [31]. A NPC is an oligomeric protein complex and contains a large aqueous channel with a diameter of approximately 9 nm [87, 88]. This channel should allow free diffusion of Ca^{2+} [87-89], mRNA [79] and molecules smaller than 40 kDa [90], whereas transport of larger molecules requires a nuclear localization signal (NLS) and energy. NPCs are anchored to the nuclear membrane via nuclear pore protein Gp210 which contains multiple Ca^{2+} binding domains facing the lumen of the NE [31]. Interestingly, the permeability of NPCs is regulated by the luminal $[Ca^{2+}]$ of the NE [91]. Gensburger and coworkers demonstrated this direct link between elevated $[Ca^{2+}]$ in the NE, caused by increased SERCA activity, and opening of the NPCs [38]. Likewise, when the NE was Ca^{2+}-depleted a conformational change displaced the central plug of the NPC, thereby blocking the pore [92], however there is no evidence that this plug blocks Ca^{2+} transport [79]. Moreover Bustamante et al. state that this plug is non-existing but instead reflects a large cargo transported between the cytosol and the nucleus [93].

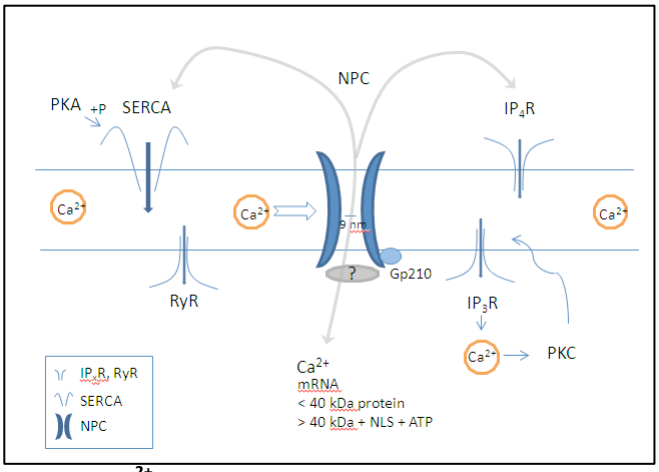


Figure 2: Ca^{2+} transport across the nuclear envelope.
In dark bleu as NPC for passive diffusion. Active uptake of Ca^{2+} into the nucleus is mediated by SERCA and IP_4R at the cytosolic membrane and RyR and IP_3R at the inner nuclear membrane.

In summary; there are two main mechanisms to change the nuclear Ca^{2+} concentration in order to stimulate or reduce Ca^{2+}-dependent transcription. These mechanisms involve passive diffusion through NPCs or via active uptake by SERCA and release via IP_3R or RyRs.

Since uptake mechanisms like SERCA and IP_4R were only identified in the outer nuclear membrane [79], the following pathway in NE Ca^{2+} homeostasis has been suggested:
Once Ca^{2+} ions are stored in the NE, stimulation of IP_3Rs or RyRs will initiate Ca^{2+} release into the nucleus. Reduction of the nuclear [Ca^{2+}] is subsequently mediated by free diffusion via the NPCs, followed by re-uptake at the cytosolic side of the NE by SERCA or IP_4R [79]. Together these mechanisms keep the nuclear Ca^{2+} concentration in a narrow range.

Nuclear targets of Calcium signalling:

Cellular Ca^{2+} concentrations are kept in a narrow range because in cardiomyocytes cytosolic Ca^{2+} homeostasis is primarily responsible for the correct handling of excitation-contraction coupling. Changes in nuclear $[Ca^{2+}]$ ($[Ca^{2+}]_n$) are able to modulate various nuclear activities, under which cell progression initiation, activation of degradative processes in apoptosis, and last but not least changes in gene expression [85,94]. Ca^{2+} induced changes in gene expression can be initiated via two general mechanisms, which are changes in transcriptional responses or chromatin density alterations.

Since oscillations in cytosolic and nuclear Ca^{2+} control transcription by distinct mechanisms, changes in a single second messenger concentration (Ca^{2+}) can results in diverse transcriptional responses [reviewed in 95,96]. Increases in the cytosolic Ca^{2+} concentration enable transcription via the Serum-response element (SRE). SRE can be activated by the Serum-response factor (SRF), PKC, Ras and several growth factors [97], however increase in nuclear Ca^{2+} is not required. Furthermore Rosen et al. demonstrated that Ca^{2+} influx through voltage-gated channels is sufficient to activate Ras, MEK, and MAP kinase Erk in neuronal PC12 cells [98]. Although, it is uncertain whether Ras activation in response to Ca^{2+} influx directly results in altered gene expression [99].
Still, Ca^{2+} does affect SRE-mediated transcription of *c-fos* by enhanced phosphorylation of SRF. Therefore SRF needs to be phosphorylated (*in vitro*) at serine 103 by CaMKII and CaMKIV, resulting in enhanced affinity of SRF for SRE [100,101].

Changes in nuclear Ca^{2+} concentrations induce transcription via the Ca^{2+}/cAMP response element (CRE). This element is found in the promoter enhancer of genes and is activated by binding of the nuclear specific Ca^{2+}-responsive transcription factor CRE binding protein (CREB) [102,103]. Binding of CREB to this site requires phosphorylation on serine 133 by cAMP dependent protein kinase A (PKA) or Ca^{2+}/CaM dependent kinase IV (CaMK-IV) [104, 105]. Phosphorylation of CREB is required for the recruitment of CREB-binding protein (CBP) to this promoter site [106]. CBP (a co-activator) subsequently links many other proteins to the transcriptional machinery at the CRE site [102].

Phosphorylation of CREB on serine 142 is thought to have a negative regulatory effect [104]. This phosphorylation is mediated by several kinases, in which the cAMP/PKA pathway is suggested to be the main modulator [107].

Ca^{2+} dependent changes in chromatin structure:

The second general mechanism in the regulation of gene expression is the control of chromatin condensation. Chromatin is composed of nucleosomes that contain histone octomers wrapped in DNA. These nucleosomes have to ability to interact, thereby creating a tight structure and prevent access of the transcriptional machinery, resulting in repressed gene expression. Post translational modifications (PTMs), such as acetylation and methylation, on conserved lysine residues can alter this chromatin density. Consequently expression of genes is determined by the histone code i.e. the composition of PTMs on histones (and especially since histone 3 is most abundant in nucleosomes) [108].

Chromatin relaxation (and expression of genes) is obtained by histone acetyltransferase (HAT)-mediated transfer of an acetyl group to a lysine residue, since addition of this group will neutralize the bond between negatively charged DNA backbone and normally positively charged histone tail. Cardiac genes are often regulated by HATs (examples are p300 and Tip60 [109, 110]) since they interact with key transcription factors like GATA4, Myocyte enhance fator-2 (MEF-2) and SRF. Moreover, Davidson et al. demonstrated that HAT inhibitors are able to prevent agonist-dependent hypertrophy in cultured cardiomyocyte [111].

Gene expression is repressed by the antagonist of HAT, the so-called Histone Deacetylases (HDACs), since removal of the acetyl groups will recover the electrostatic attraction, and might facilitate conjugation of other PTMs like methyl or ubiquitin groups. These modifications alter gene expression by creating new docking sites for co-activators or co-repressors [112].

(De-)Acetylation not only regulates chromatin density, but also non-histone proteins such as GATA4 and MEF2 [113] are regulated by this mechanism [114]. Interaction of HDACs to members of the MEF2 family of transcription factors [115,116] regulates cardiac gene expression. Especially since MEF2 was shown to be upregulated in response to pathological stress to the heart [117,118] resulting in dilated cardiomyopathy in mice [119].

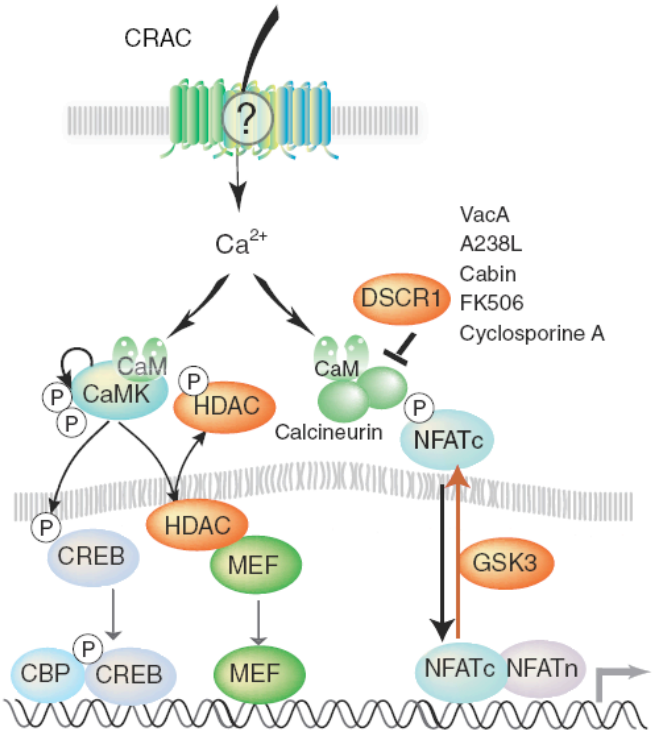


Figure 3: An overview of three important pathways with respect to gene transcription, that may be activated in response to Ca^{2+} transients. Activators and inhibitors are indicated. Adapted from Gallo 2006.

Ca^{2+} can be held responsible for the changes in gene expression, since activation of HDACs depends on phosphorylation mediated by kinase such as CaMKII [120]. CaMKII isoforms delta and gamma are highly expressed in heart [121], and play a critical role in pathological remodeling of the heart [122]. Moreover, activation of the endogenous CaMKII signaling is associated with cardiac hypertrophy in mouse models overexpressing CaM in cardiac cells [123].

NFAT

One example of a well-studied family of transcription factors that are regulated by cytosolic Ca^{2+} transients is the Nuclear factor of activated T-cells (NFAT) family. The NFAT family of transcription factors comprises 4 isoforms (NFAT1-4). Most isoforms are constitutively expressed in the cytosol as transcriptionally inactive phospho-proteins. Phosphorylated NFAT is localized in the cytoplasm since both nuclear localization sequences (NLS) are masked by phosphate groups attached to serines in the SP repeat and serine rich region [124].

During EC- or ET-coupling cytosolic Ca^{2+} levels increase (via CICR) and the Ca^{2+}-CaM-dependent serine/threonine specific phosphatase 2B, also known as Calcineurin (Cn) [125,126], activates NFAT by removing these phosphate groups [107,127-130]. Dephosphorylation relieves the suggested intra-molecular interaction and exposes the NLSs allowing NFAT translocation to the nucleus [124,130]. NFAT translocation is the rate-limiting step in Cn-NFAT signaling module. Moreover, NFAT re-phosphorylation in the cytoplasm is extremely slow, allowing nuclear-ready-NFAT to be transported to the nucleus for several minutes. In addition multiple Ca^{2+} oscillations will build up the level of dephosphorylated NFAT in the cytoplasm [131]. Therefore dephosphorylated (nuclear-ready) NFAT serves as a working memory in Ca^{2+} signaling [131].

This direct link between Ca^{2+} oscillation and Cn-NFAT as a signaling pathway was determined in cardiomyocytes by the discovery of NFAT4 associating with the zinc finger transcription factor GATA4 [132]. GATA4 is suggested to regulate expression of genes involved in myocardial function and differentiation [133].

The activation of NFAT4 is tightly regulated since activation of Cn is regulated by Ca^{2+} fluxes originating from the SR [107]. Above all, Cn controls both transcriptional activation and intracellular Ca^{2+} concentrations. This later effect is mediated by the dephosphorylation of PLB, the inhibitor of SERCA pumps [134].

Once NFAT enters the nucleus, it is responsible for the initiation of transcription of several early response genes such as; IL-2, IL-4 [135,136], CD40 ligand and Fas ligand [124,137] as well as Ca^{2+} signalosome proteins like Endothelin-1, IP_3R1, NFAT2 and DSCR1 [4]. Nuclear export of NFAT subsequently requires phosphorylation of the conserved serines by glycogen synthase kinase-3 (GSK3) [124,138].

Interestingly, physiological stimuli, such as exercise, as well as pathological excitations, like responses to hypertension or myocardial infarction injury, can induce alterations in contractility that are associated with altered Ca^{2+} transients [126].

Yet, it is unclear if Cn activity is regulated by stress-induced increases in contractile Ca^{2+}. However, Wilkins et al. and Molkentin et al. demonstrated that Cn activation is capable of inducing pathologic cardiac hypertrophy and deleterious remodeling of the heart by activation of NFAT [139, 140]. Importantly, this Cn-NFAT signaling pathway does not seem to be activated during exercise-induced hypertrophy [141] or pregnancy, even though contractile Ca^{2+} is increased.

Heart failure; Causes of hypertrophy.

As described in the previous sections, intracellular Ca^{2+} transients are important in regulation of many aspects of life, ranging from embryogenesis, cellular functioning and differentiation to programmed cell death [142]. The cytosolic Ca^{2+} concentration is ~100 nM in resting cardiomyocytes. However, in response to an action potential CICR is activated and the intracellular Ca^{2+} concentration will rise to 1-2 µM [15].
To be able to decrease this temporary high [Ca^{2+}] SERCA pumps and NCX are activated to store excess Ca^{2+} in intracellular and extracellular compartments respectively.

Cardiomyopathies such as cardiac hypertrophy are associated with abnormalities in Ca^{2+} handling. For instance, alterations in the amplitude or duration of Ca^{2+} transients (induced by hypertrophic stimuli) are responsible for the phenotypic remodeling of cardiomyocytes [4]. Moreover, upregulation of proteins involved in Ca^{2+} handling, contractility and energy metabolism of the fetal heart cause dedifferentiation and have been linked to cardiac hypertrophy [4, 132].

Since the SR in cardiomyocytes is a major source for contractile Ca^{2+}, many studies pinpoint to SR-dysfunction (especially SERCA) as the major cause for altered Ca^{2+} homeostasis [4].
Studies by Matsui and Qi respectively demonstrate a significant decrease in cardiac SERCA2a expression in animal models of cardiac hypertrophy [143, 144] as well as in human tissue samples of end-stage failing heart [145]. In addition, NCX activity appeared to be increased in cardiomyopathic hamster, human failing myocardium [146], as well as in mice over-expressing LTCC (in an attempt to counteract the increased Ca^{2+} influx) [4].
Interestingly, cardiac function of rats characterized with low endogenous cardiac SERCA levels, could be restored by adenovirus mediated gene transfer of SERCA2a [147].
Under normal conditions, SERCA activity is inhibited by PLB. Phosphorylation of PLB (by PKA or CaMKII) blocks this inhibition (i.e. SERCA activity is stimulated). Of note, genetic deletion of PLB prevented functional defects in mouse models of dilated cardiomyopathy [148].

As mentioned previously, SERCA2a expression is reduced in heart failure and hypertrophy, and is often accompanied by a downregulation of RyR2 expression [142]. Moreover, a defect in the coupling between remaining RyRs and LTCC has been reported as one of the underlying causes for hypertension and hypertrophy in rat hearts [149]. Reduced RyR expression itself could be compensated by upregulation of IP_3-mediated signaling and IP_3Rs expression [150].

RyRs in the heart are composed of four RyR_2 peptides and four FKBP12 proteins. The interaction of FKBP12 with RyR2 reduces channels activity [151]. Moreover, severe cardiac hypertrophy was found in mice deficient of FKBP12 [152]. FKBP12 also dissociates from RyR after PKA phosphorylation of the RyR receptor, thereby increasing the open probability and Ca^{2+} sensitivity of the channel [153].

Immunosuppressive drugs like Rapamycin or FK506, disrupt the interaction between RyR and FKBP12 and thereby stimulate SR Ca^{2+} release. Hence a suggested side-effect of these immunosuppressive drugs is hypertrophy or heart failure due to increased muscle contraction [154]. However, mice deficient of RyR2 receptors die early in embryonic development (approximately day 10) as a result of morphologic abnormalities of the heart tube [155].

Cardiac hypertrophy can also be caused by overexpression of Cq [156]. Although the storage capacity of the SR is increased by overexpression of Cq, stored Ca^{2+} ions are not available for CICR during EC-coupling resulting in reduced Ca^{2+} transient amplitude. Moreover, overexpression of Cq in mouse cardiomyocytes results in downregulation of proteins involved in CICR such as RyR2, TRD and JCN [4]. TRD1 overexpression was compensated by a similar principle, *i.e.* decreased expression of RyR2 and JCN.

Of course, hypertrophy is not only caused by abnormalities in SR Ca^{2+} handling, instead it is often caused by multiple alterations. Likewise, mutations in Ca^{2+} handling proteins do not only affect cardiac remodeling, but can cause a wide range of diseases (an overview in [15] (table 1)).

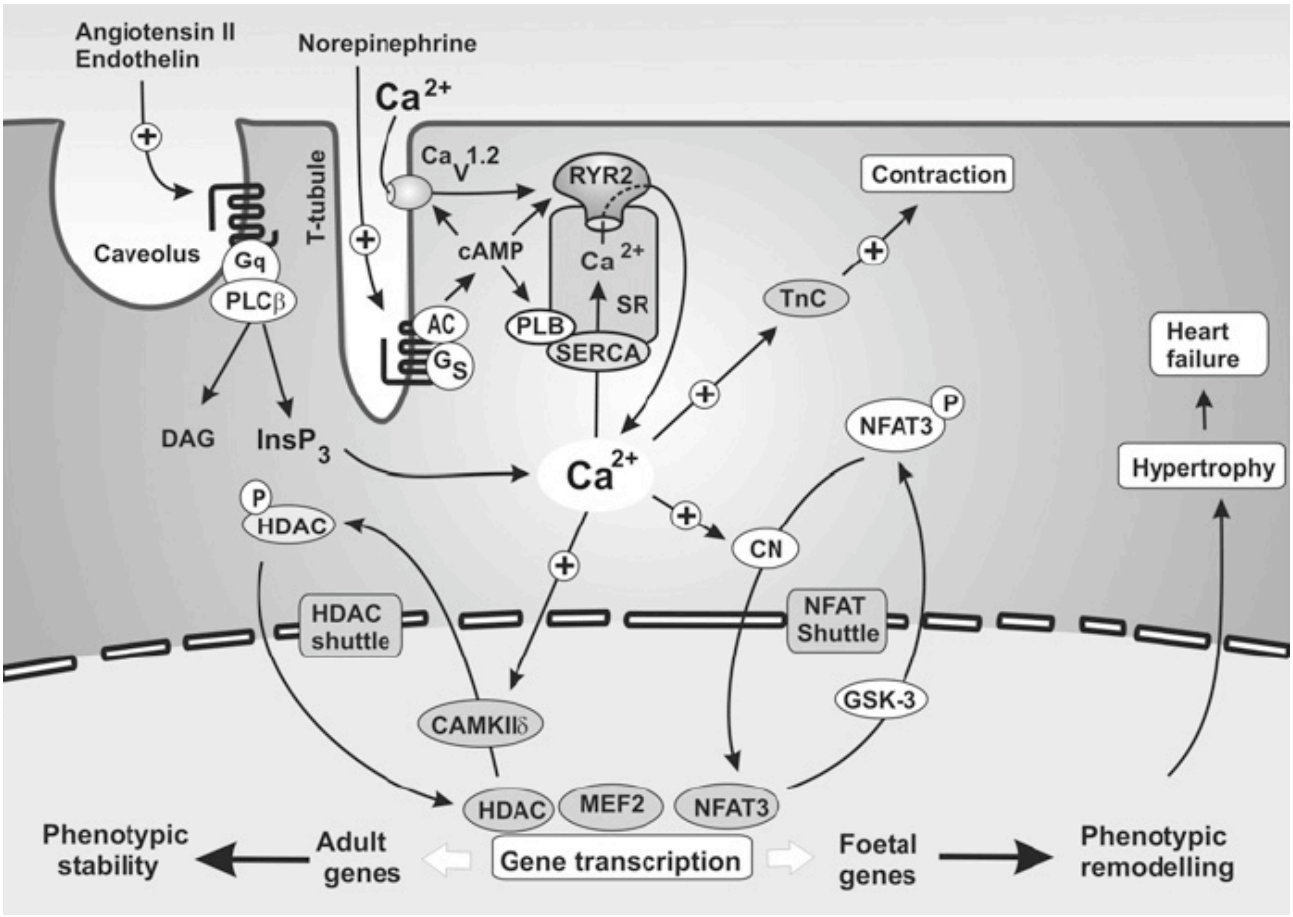


Figure 4: In summary. An overview of the cytosolic Ca^{2+} uptake and Ca^{2+} release mechanisms, NFAT shuttle and other effectors in gene expression. Adapted from [4].

Discussion

Calcium is a very important ion since one of its functions is to directly initiate physical contraction of cardiomyocytes. Ca^{2+} is therefore essential in the regulation of cardiac rhythm. Interestingly, intracellular Ca^{2+} does not only regulate this specific physiological response, instead Ca^{2+} is widely used as a second messenger. Changes in the amplitude, duration and frequency of Ca^{2+} transients are used to control a divers set of responses ranging from embryogenesis and cell differentiation to processes in programed cell death. These mechanisms are either controlled by Ca^{2+}, since it functions as a direct co-factor in the transcriptional machinery, or because Ca^{2+} transients activate certain kinases and phosphatases that subsequently activate transcription factors. Examples of transcription factors that are regulated by Ca^{2+} are CREB (after cytosolic Ca^{2+} increase), SRF (in response to nuclear Ca^{2+} alterations) and last but not least the regulation of transcriptional control by NFAT has been described.

Furthermore, the influence of Ca^{2+} on chromatin condensation and thereby the accessibility for the transcriptional machinery, has been discussed in this review. As described, both acetylation by HATs and deacetylation by HDACs are regulated by Ca^{2+}. Although not defined, other modifications, like ubiquitin or methyl groups, can be added or removed from histones in response to Ca^{2+} fluctuations.

Since Ca^{2+} has these many functions with the same cell type (here we mainly discussed cardiomyocytes) it is conceivable that tight regulation is a top priority! If regulation fails, diseases such as cardiac hypertrophy and eventually heart failure may occur. And one must keep in mind that heart diseases are the major cause of human morbidity and mortality [4].

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